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Hormonal Regulation of Epidermal Tumor Development

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The effects of hormones and hormone-like substances such as thyroxine, estradiol, hydrocortisone, calcitonin, prostaglandins ($F_{2\alpha}$, A_2) or hormone deficiency (hypophysectomy, gonadectomy) on the development of carcinomas in mice and rats induced by 3-methylcholanthrene were investigated.

Thyroxine, $PGF_{2\alpha}$, calcitonin, and estradiol markedly enhanced the development of squamous cell carcinomas and basal cell carcinomas; an inhibition occurred following hydrocortisone, hypophysectomy, and gonadectomy. DNA labeling using [^{3}H]-thymidine and radioautography revealed that DNA synthesis in neoplastic cells is also markedly influenced by the same hormones.

Tumor histology and ultrastructure are profoundly affected and an increase of epithelial pearls, hyperchromatic basophilic cells, tonofilaments, polyribosomes, and lysosomes is observed following thyroxine and $PGF_{2\alpha}$. An increase of keratinization and intramitochondrial granules occurred with estradiol and increased sclerosis followed calcitonin. A marked reduction of cell organelles occurred after hydrocortisone, hypophysectomy, and gonadectomy. Scanning electron microscopy shows salient changes in tumor cytoarchitecture and cell surfaces (blebs, ruffles).

These findings demonstrate that hormones can change the development of carcinomas, DNA synthesis as well as their cellular differentiation and consequently may be important modulators of epidermal carcinogenesis.

The study of factors which can regulate the onset and development of epidermal tumors is of special interest for carcinogenesis. It is well known that hormones and hormone-like substances are potent factors in controlling cell division and proliferation. For this reason extensive investigations regarding the role of hormones on tumor behavior have been carried out since the beginning of this century [1]. However, most of the investigations regarding the role of hormones such as estrogens, progesterone, prolactin, or androgens were carried out on the development of mammary cancers or prostatic tumors and it was found that they can significantly change the neoplastic growth in these target tissues [2,3]. Previously, it was found that topical application of 3-methylcholanthrene (3-MCA) on the dorsal skin consistently induced 2 different types of tumors: squamous cell carcinoma in mice and basal cell carcinoma in rats. Concomitant administration of $PGF_{2\alpha}$ and PGE_2, but mostly $PGF_{2\alpha}$, markedly shortened the latency period and thus enhanced the occurrence of squamous cell carcinoma in mice [4]. Administration of dexamethasone or fluocinolone acetonide inhibits tumor initiation by both 3-MCA and 7,12-dimethylbenz (a)anthracene (DMBA) on mouse skin and this may be related to their ability to inhibit the aryl hydrocarbon hydroxylase (AHH) and DNA synthesis [5].

Most of the investigations were carried out regarding the hormone effects on epithelial hyperplasia and papillomas; however, there are no studies regarding the role of hormones on epidermal carcinomas. Since squamous cell carcinomas and basal cell carcinomas are similar to those occurring in humans, they present an interesting experimental model for the study of epidermal carcinogenesis and its responsiveness to hormones.

The present studies were carried out in order to find out if the hormones, which are widely used for the treatment of cancers of other tissues, are critical factors in controlling epidermal tumor morphology, their cellular differentiation and DNA synthesis.

MATERIALS AND METHODS

Experimental Animals

Male albino Swiss mice (≈2 mo old, cesarean-origin, barrier-sustained) weighing between 20 and 25 gm and male albino Sprague-Dawley rats (≈1 mo old, cesarean-origin, barrier-sustained) weighing between 100 and 125 gm, were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). The animals were divided into 10 experimental groups (10 mice and 10 rats/group)(see Table I).

MCA and Hormone Preparations

3-MCA was dissolved in acetone (0.4%) and applied with a pipette on a marked and shaved region on the dorsal skin (Table I). $PGF_{2\alpha}$ and PGA_2 were dissolved in 0.2 ml of absolute ethanol and 0.02% sodium carbonate (1:9). L-thyroxine (Synthroid) was dissolved in saline solution (0.9%). Estradiol valerate (Delestrogen) was dissolved in sesame oil. Synthetic Salmon Calcitonin (Calcimar) was dissolved in a 16% gelatin solution. Gonadectomy was bilateral and performed according to the standard procedure. Hypophysectomy was performed by parapharyngeal route. Completeness of hypophysectomy was assessed at autopsy using a magnifier (× 10). The injection sites were far from the site of MCA applications. The hormones and 3-MCA were administered throughout the experiments. Tumors were counted monthly and their size was measured in both mice and rats. At the end of the experiments (12 mo for mice and 16 mo for rats) and 2 hr prior to sacrifice, 5 mice and 5 rats from each experimental group received concomitantly with MCA and hormones, 7 μCi[^{3}H]-thymidine/gm bwt, im (sp. act, 28.5 Ci/mmol) for the study of DNA synthesis. The time of sacrifice was determined by the maximum development of carcinomas; however, mice and rats (groups VI and X), which were treated as the other groups, but developed only mild epidermal hyperplasia (tumor-free) were sacrificed at the same time. Two hours was selected for isotope studies because it was found that some hormones such as calcitonin and PG exert their maximum effect on cell metabolism in that time [6,7].

DNA Labeling

Pulse labeling of DNA content in control epidermal and neoplastic cells was performed on at least 5–6 specimens which were removed from each experimental group, dissected, homogenized using a Potter

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Abbreviations:
AHH: aryl hydrocarbon hydroxylase
bwt: body weight
DMBA: 7, 12-dimethylbenz(a)anthracene
MCA: methylcholanthrene
GTA: glutaraldehyde
PG: prostaglandin
sp. act: specific activity

TABLE I. *Induction of epidermal carcinomas and hormones*

Group	Treatment	Mice[a] Dose	Mice[a] Administration	Rats[b] Dose	Rats[b] Administration
I	Controls & diluent	0.2 ml	Locally (3 × weekly)	0.2 ml	Locally (3 × weekly)
II	MCA[c] & diluent	0.2 ml 0.2 ml	Locally & (3 × weekly)	0.2 ml 0.4% & 0.2 ml	Locally & (3 × weekly)
III	MCA & $PGF_{2\alpha}$	0.2 ml MCA & 10 μg $PGF_{2\alpha}$	Locally & (3 × weekly) im.	0.2 ml MCA & 50 μg $PGF_{2\alpha}$	Locally & (3 × weekly) im.
IV	MCA & PGA_2	0.2 ml MCA & 10 μg PGA_2	Locally & (3 × weekly) im.	0.2 ml MCA & 50 μg PGA_2	Locally & (3 × weekly) im.
V	MCA & thyroxine (T_4)	0.2 ml MCA & 2 μg T_4	Locally & (3 × weekly) im.	0.2 ml MCA & 5 μg T_4	Locally & (3 × weekly) im.
VI	MCA & hydrocortisone (HC)	0.2 ml MCA & 1 mg HC	Locally & (3 × weekly) im.	0.2 ml MCA & 2.5 mg HC	Locally & (3 × weekly) im.
VII	MCA & estradiol (E)	0.2 ml MCA & 200 μgE	Locally & (3 × weekly) im.	0.2 ml MCA & 1 mg E	Locally & (3 × weekly) im.
VIII	MCA & calcitonin (C)	0.2 ml MCA & 2 MRCU[d] C	Locally & (3 × weekly) im.	0.2 ml MCA & 5 MRCU C	Locally & (3 × weekly) im.
IX	MCA & gonadectomy	0.2 ml MCA	Locally (3 × weekly)	0.2 ml MCA	Locally (3 × weekly)
X	MCA & hypophysectomy	0.2 ml MCA	Locally (3 × weekly)	0.2 ml MCA	Locally (3 × weekly)

[a] All experiments in mice were carried out for 12 mo.
[b] All experiments in rats were carried out for 16 mo.
[c] MCA (methylcholanthrene).
[d] MRCU (Medical Research Council Units).

homogenizer and washed several times with 0.4 M perchloric acid, ethanol and ether [8]. In control experiments it was checked that the acid-soluble radioactivity was completely removed (or washed out) by this procedure. Measurements were performed with a nuclear liquid scintillation system (Isotope-300; Searle Analytic) with an efficiency of 40% and with the use of tritium as an internal standard. DNA radioactivity was expressed as % of controls.

Light Microscopic Autoradiography

The specimens were fixed in Bouin's solution for 12–24 h, then dehydrated and embedded in paraplast. Sections (5 μ thick) were stained with H & E, covered with Kodak Nuclear Emulsion NTB_2 and exposed for 14 days at 4°C in a dark room. The filmed sections were processed in D_{19} Kodak developer and rapid fixer, washed, dehydrated, and mounted. Autoradiograms were examined under Zeiss microscope at × 400 (objective × 40; ocular × 10) and quantitative estimation was performed by counting the labeled cells from 2,000 consecutive epithelial nucleated cells in the basal layers of control mice or in the proliferative compartments following treatment with 3-MCA and hormones. The percentage of labeled cells was also recorded.

Electron Microscopy

Specimens from tumors of mice and rats as well as from dorsal skin of controls were removed, diced, fixed for 24 hr in 3% cacodylate buffered glutaraldehyde, postfixed for 1½ hr in 1% phosphate osmium tetroxide, dehydrated in ascending series of ethanol and propylene oxide, and embedded in a mixture of Epon:Araldite. Thin sections (≈600 Å) were cut using LKB Ultrotome III equipped with a diamond knife mounted on grids and stained with aquaeous solutions of uranyl acetate and lead citrate.

Electron Microscopic Autoradiography

Grids with sections were covered with Ilford L_4 Nuclear Emulsion diluted in water 1:2 using a wire loop procedure, and exposed for a period of 2–3 mo in dark boxes with drierite at 4°C, then developed in Microdol-X, fixed, washed, and short stained with uranyl acetate and lead citrate. Sections were examined under HS-8 Hitachi electron microscope at 50 kv [9].

Scanning Electron Microscopy (SEM)

Larger specimens from tumors were fixed in 3% cacodylate buffered GTA, dehydrated, and embedded using critical point method with CO_2. Specimens coated with gold (≈200 Å) were viewed in ETEC Scanning electron microscope at 20 kv.

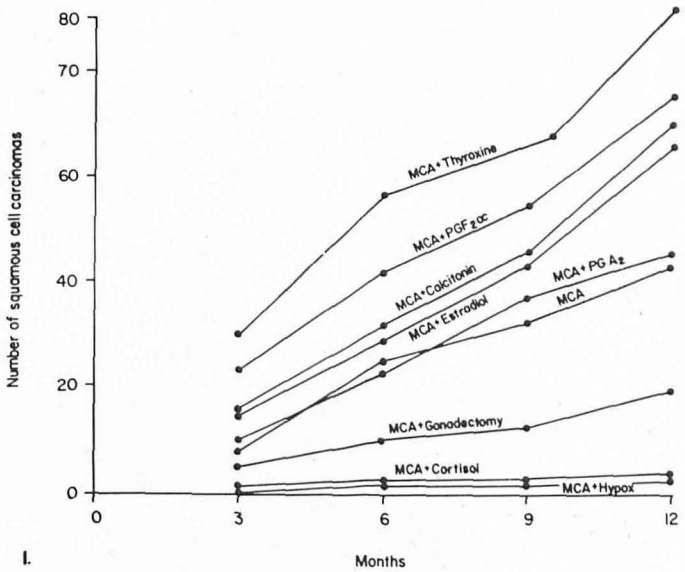

FIG 1. The onset and development of squamous cell carcinomas in mice treated with 3-methylcholanthrene (*MCA*) and different hormones.

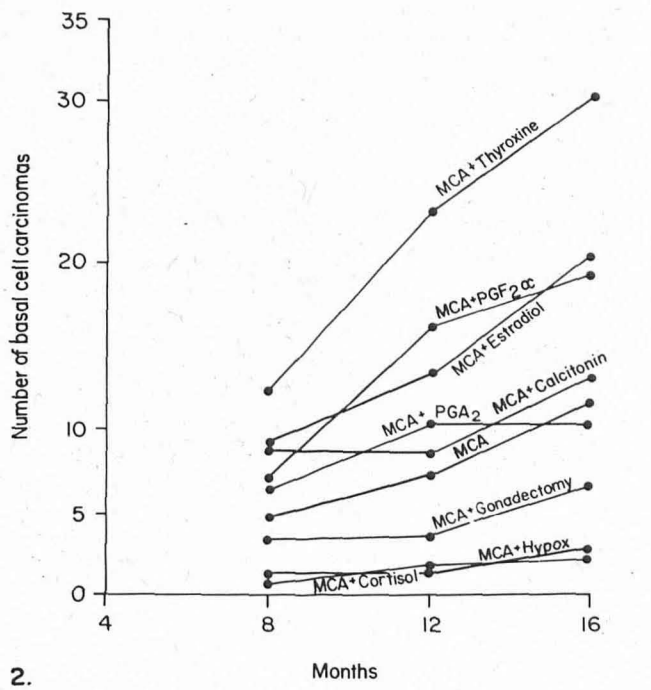

FIG 2. The onset and development of basal cell carcinomas in rats treated with 3-methylcholanthrene (*MCA*) and different hormones.

RESULTS

Tumor Incidence and Development

Multiple large and keratinized tumors, sometimes necrotic and hemorrhagic which coalesce and occupy the entire middorsal region are seen in mice starting at 3 mo. These tumors are squamous cell carcinomas. Their onset and development was markedly affected by hormone administration, hypophysectomy, and gonadectomy. The curves of mouse tumorigenesis at 3, 6, 9, and 12 mo were recorded (Fig 1). Rats developed different types of tumors. The tumors occurred later, starting at 8 mo, as small pinkish and pearly nodules with waxy rolled borders. They slowly grow, then centrally ulcerate and coalesce forming clusters of tumors which can reach 2–3 cm in diameter. These are basal cell carcinomas. Hormone administration, gonadectomy, and hypophysectomy significantly affect the onset and development of these tumors. Curves of rat tumorigenesis at 8, 12, and 16 mo were also recorded (Fig 2).

DNA Labeling

DNA labeling expressed as % of controls showed notable changes in the neoplastic nuclei of squamous cell carcinoma as compared to hormone-treated, castrated, and hypophysectomized mice. Thus, a marked increase of DNA radioactivity was observed after thyroxine, $PGF_{2\alpha}$ (Groups V, III), and estradiol (VII) followed by that of calcitonin (VIII) and PGA_2 (IV) and a significant decrease after hydrocortisone, gonadectomy, and hypophysectomy (Groups VI, IX, and X)(Fig 3). DNA radioactivity is also increased in rats with basal cell carcinoma and thyroxine, estradiol, and $PGF_{2\alpha}$-treated (Groups V, VII, and III). A marked decrease was observed in rats with tumors and cortisol-treated, gonadectomized, and hypophysectomized (Groups VI, IX, and X)(Fig 4).

Tumor Histopathology

Tumor cytology revealed interesting findings regarding cell growth, differentiation, predominance, and variance of dark and light cells following hormone administration. Thus, in mice following MCA application, a typical invasive structure, with characteristic epithelial pearls is visible; a mild dermal infiltration occurred. These are well-differentiated squamous cell carcinomas (grade I). In MCA-treated rats the tumors are mainly basal cell carcinomas, composed of cords with small, hyperchromatic basal cells and large nuclei. A more invasive squa-

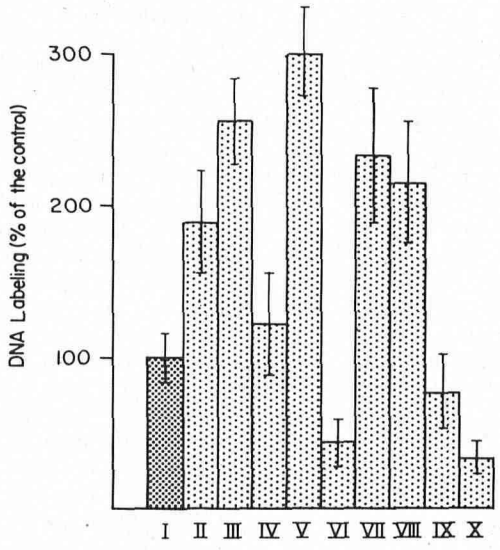

FIG 3. DNA labeling in squamous cell carcinomas: *I* (control mice, taken as 100); *II* (3-methylcholanthrene [MCA]); *III* (MCA & $PGF_{2\alpha}$); *IV* (MCA & PGA_2); *V* (MCA & thyroxine); *VI* (MCA & hydrocortisone); *VII* (MCA & estradiol); *VIII* (MCA & calcitonin); *IX* (MCA & gonadectomy); *X* (MCA & hypophysectomy).

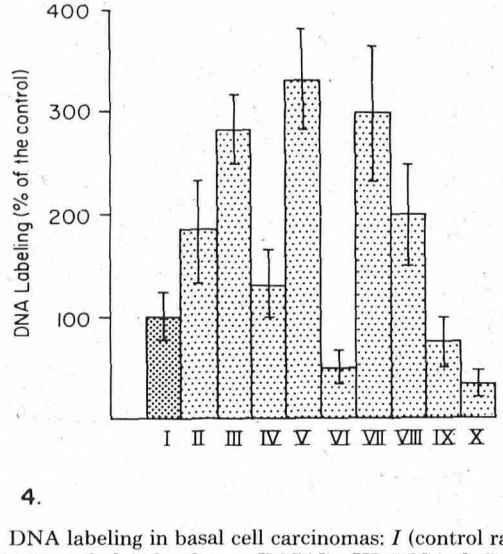

FIG 4. DNA labeling in basal cell carcinomas: *I* (control rats, taken as 100); *II* (3-methylcholanthrene [MCA]); *III* (MCA & $PGF_{2\alpha}$); *IV* (MCA & PGA_2); *V* (MCA & thyroxine); *VI* (MCA & hydrocortisone); *VII* (MCA & estradiol); *VIII* (MCA & calcitonin); *IX* (MCA & gonadectomy); *X* (MCA & hypophysectomy).

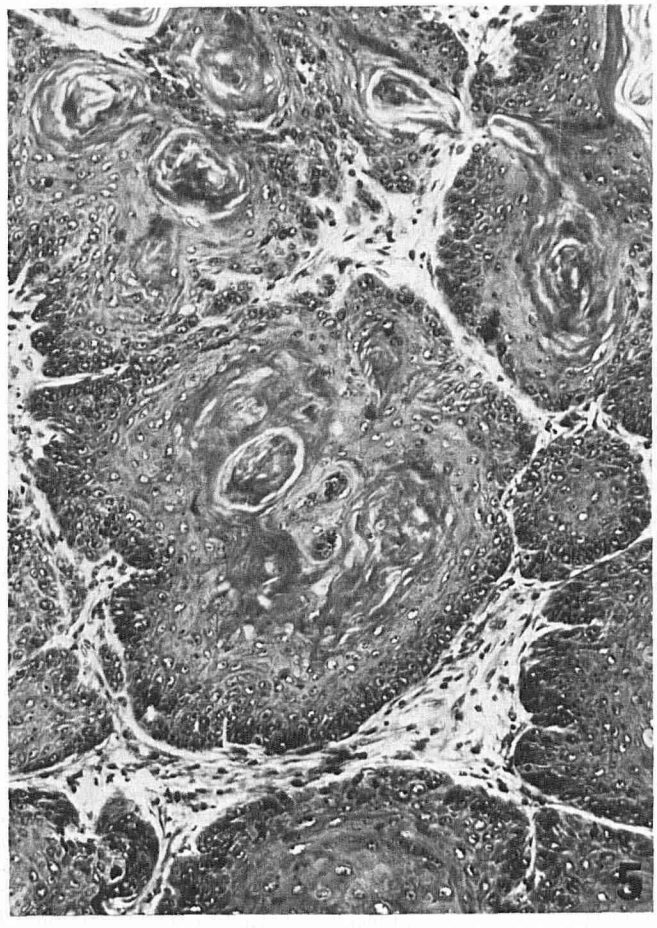

FIG 5. Invasive squamous cell carcinoma originating from hyperplastic epidermis; epithelial pearls and mild dermal infiltration can be seen in a mouse treated with 3-methylcholanthrene & $PGF_{2\alpha}$ (H & E × 400).

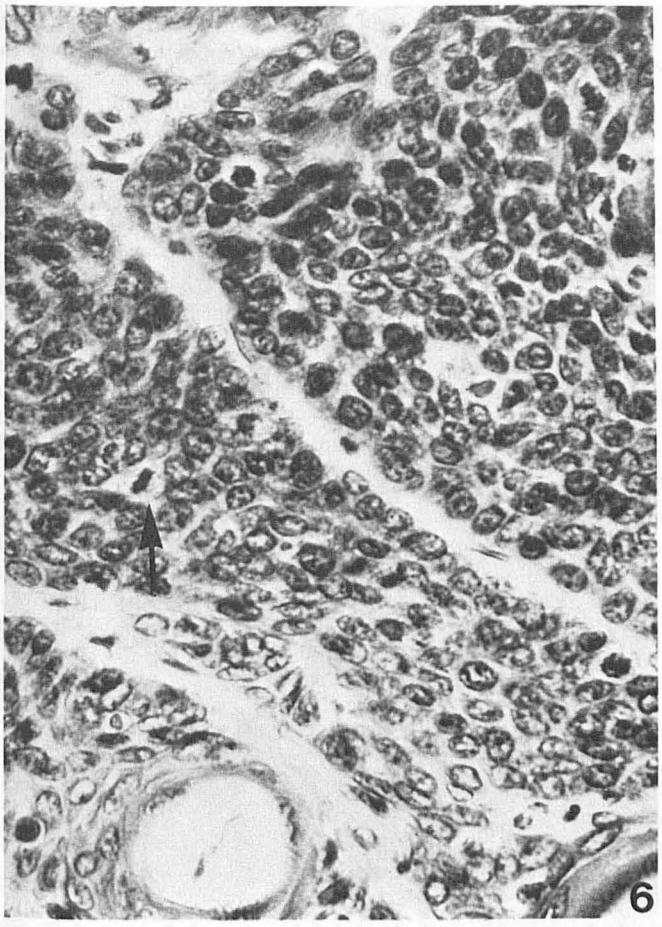

FIG 6. Squamous cell carcinoma exhibiting a massive cord-like structure with few atypical epithelial pearls, and mitotic cells (*arrow*) in a mouse treated with 3-methylcholanthrene & thyroxine (H & E × 800).

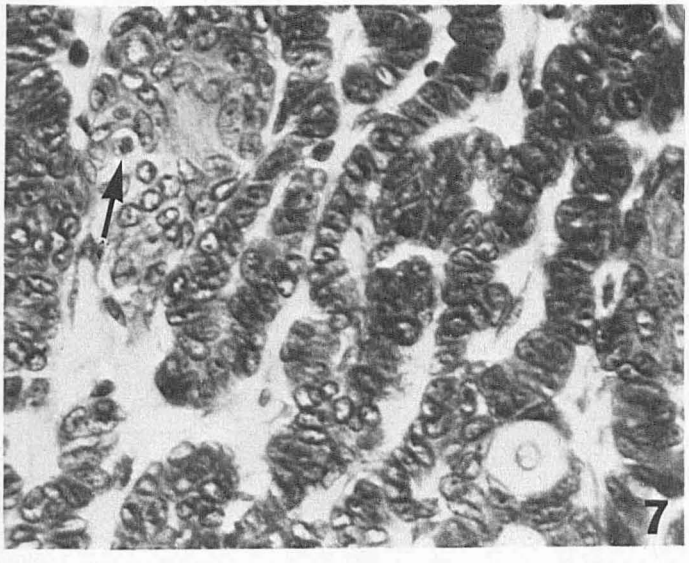

FIG 7. Solid basal cell carcinoma occurring in a rat treated with 3-methylcholanthrene and thyroxine. Cord of basophilic cells occupy all areas, separated by small vessels and connective tissue. Basophilic cells are arranged in a palisadic pattern. Multinucleated and mitotic cells are visible (*arrow*) (H & E × 800).

mous cell carcinoma occurred in MCA and $PGF_{2\alpha}$-treated mice composed of several tumor masses with numerous confluent horn or epithelial pearls and few sheets of atypical squamous cells (Fig 5). An interesting cellular pattern occurred in rats

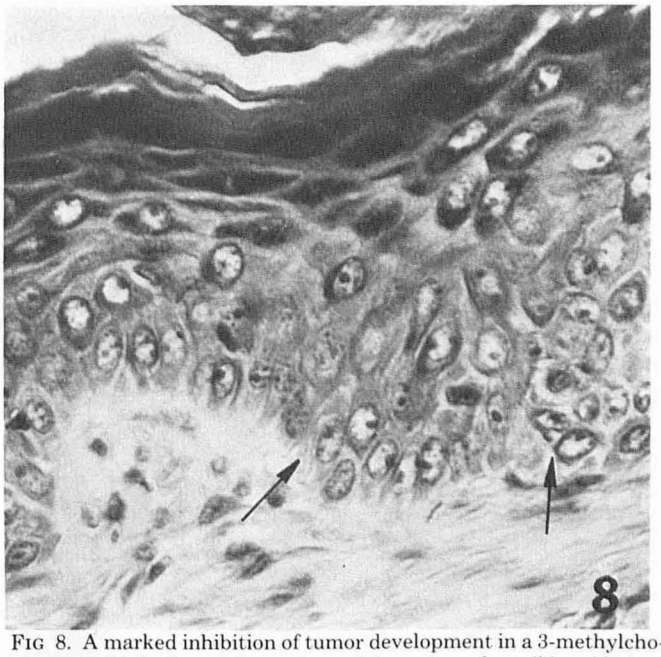

FIG 8. A marked inhibition of tumor development in a 3-methylcholanthrene and hydrocortisone treated mouse; only mild epidermal hyperplasia with vacuolated cells and fragmented collagen fibers can be seen (*arrows* indicate the site where tumor used to be) (H & E × 800).

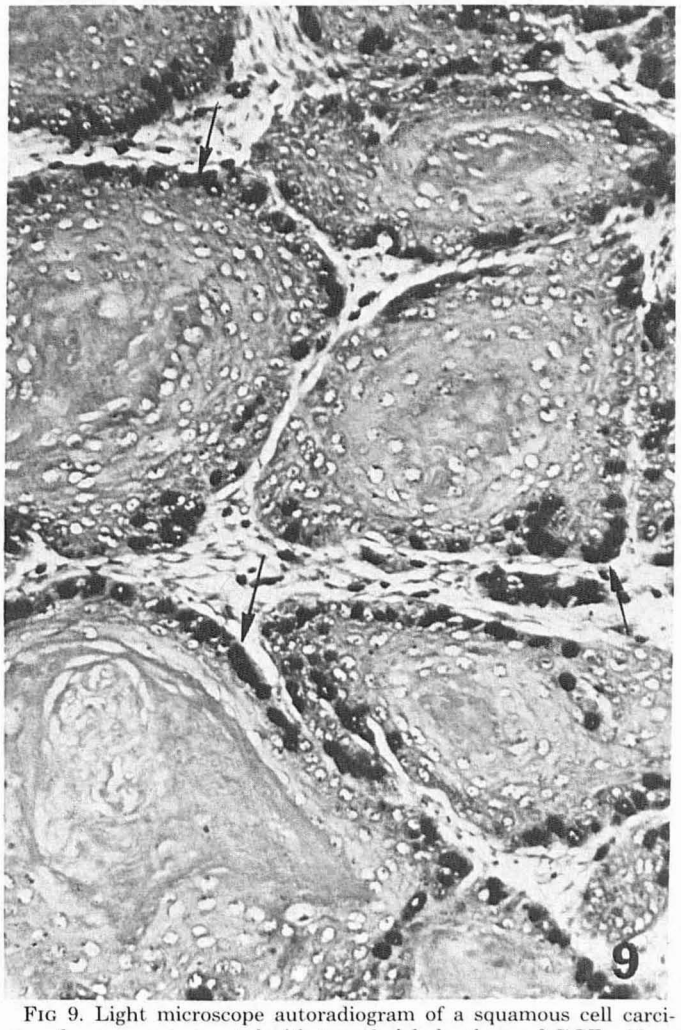

FIG 9. Light microscope autoradiogram of a squamous cell carcinoma from a mouse treated with 3-methylcholanthrene & $PGF_{2\alpha}$. Note the heavy labeled cells only in the basal proliferative compartments (*arrows*). No reaction can be seen over the epithelial pearls. Kodak nuclear NTB_2 emulsion, exposed 14 days (H & E × 800).

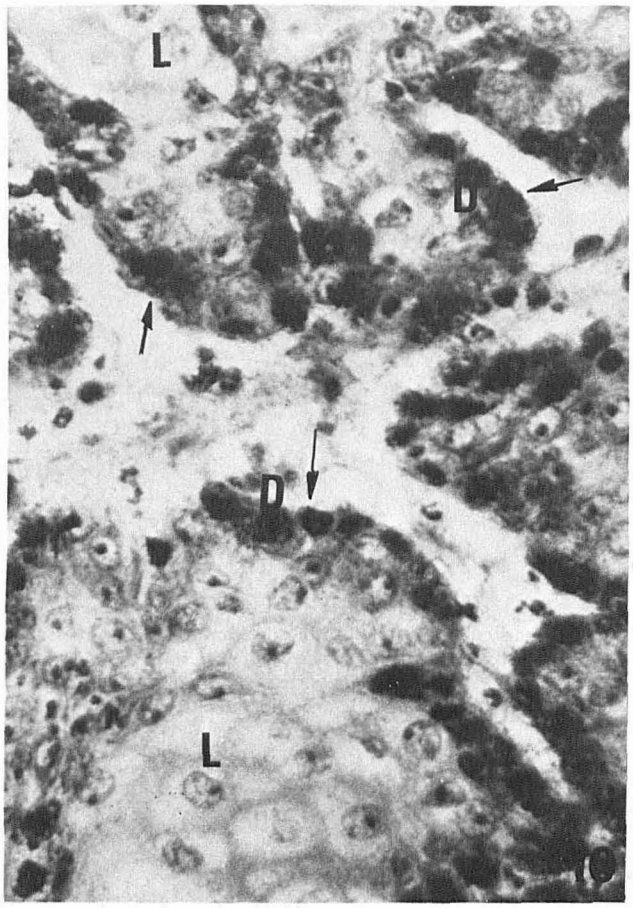

FIG 10. Light microscope autoradiogram of a basal cell carcinoma from a rat treated with 3-methylcholanthrene & $PGF_{2\alpha}$. Two distinct cell compartments can be seen: one compartment of dark and heavily labeled cells (*D*)(*arrows*), the other of light and poorly labeled cells (*L*). Kodak NTB_2 nuclear emulsion, exposed for 14 days (H & E × 800).

TABLE II. *Autoradiograms: Percentage of neoplastic cells labeled with [^{3}H]-thymidine in epidermal carcinomas of mice and rats*

Treatment	Squamous cell carcinoma		Basal cell carcinoma	
	No. of labeled cells/total No. of cells	Percentage	No. of labeled cells/total No. of cells	Percentage
Controls & diluent	166/2000	8.30[a]	124/2000	6.20[a]
MCA & diluent	401/2000	20.05	363/2000	18.15
MCA & $PGF_{2\alpha}$	785/2000	39.25[a]	696/2000	34.80[a]
MCA & PGA_2	500/2000	25.00	480/2000	24.00
MCA & Thyroxine	980/2000	49.00[a]	870/2000	43.50[a]
MCA & Hydrocortisone	108/2000	5.40	100/2000	5.00
MCA & Estradiol	670/2000	33.50	610/2000	30.50
MCA & Calcitonin	615/2000	30.75	550/2000	27.50
MCA & Gonadectomy	171/2000	8.55[c]	142/2000	7.10[b]
MCA & Hypophysectomy	135/2000	6.75[b]	110/2000	5.50[c]

[a] Statistically significant ($P < 0.001$) from the respective controls.
[b] Statistically significant ($P < 0.005$) from the respective controls.
[c] Not significant.

treated with MCA and $PGF_{2\alpha}$. The neoplastic cords are composed of 2 compartments; large areas of dark cells alternating with clear cells. Dark cells are smaller with rich chromatin nuclei (Fig 10). Only a moderate tumor formation is seen in MCA and PGA_2 treated mice and rats.

A different histologic type of tumor occurred in MCA and thyroxine-treated mice, mostly composed of cellular cords with poor differentiated or anaplastic squamous cells; mitotic cells can also be seen (Fig 6). An intense stimulation of tumor development and cellular pattern occurred in MCA and thyroxine-treated rats. The entire area is occupied by cords composed of small, hyperchromatic basal cells, with enlarged nuclei; multinucleated cells are also visible. These are solid basal cell carcinomas (Fig 7). Neoplastic cells are disposed mostly in a palisading pattern separated by capillaries and strands of collagen fibers; sometimes a fasciculate pattern (similar to adrenal cortex) or organoid (similar to liver) can also be seen. A more keratinized type of tumor occurred in MCA and estradiol-treated mice and rats. Most of the tumor masses are replaced by keratin layers with few horn or epithelial pearls. These are advanced keratinized squamous cell carcinomas and keratotic basal cell carcinomas. A different type of tumor also occurred in MCA and calcitonin-treated mice. The tumor masses are fragmented by sclerosis. A more advanced sclerotic pattern is visible in MCA and calcitonin-treated rats. There are only scattered tumor cells which are separated by large areas of sclerosis with infiltrative cells and blood vessels. These are sclerosing basal cell carcinomas. A moderate inhibition of tumor formation is observed in MCA-treated and gonadectomized mice and rats. A marked inhibition of tumor development

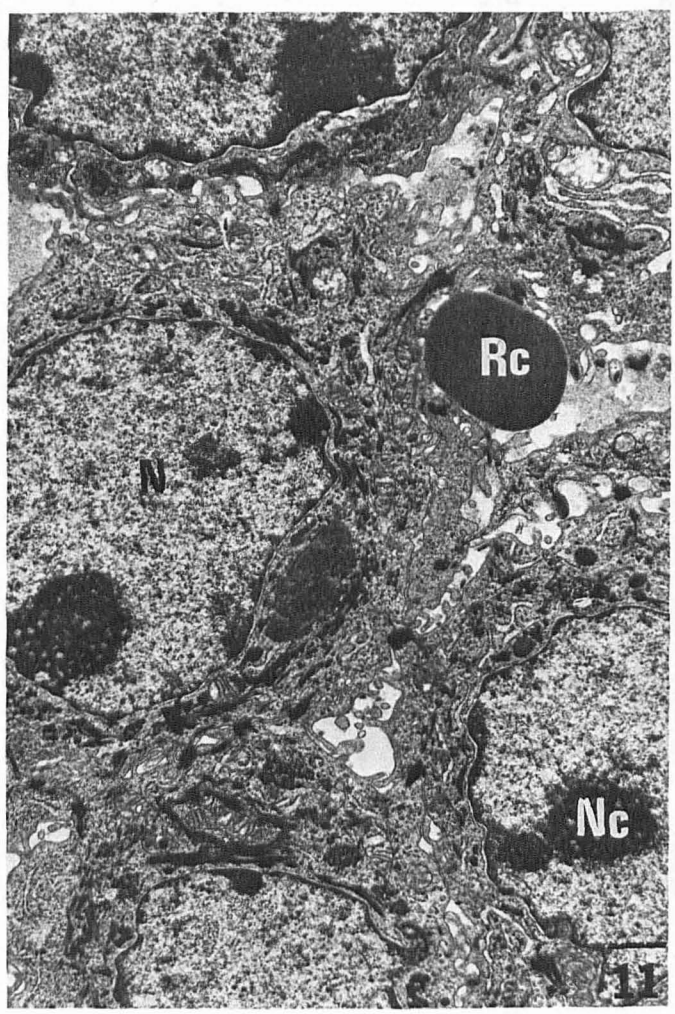

FIG 11. Squamous cell carcinoma (3-methylcholanthrene & $PGF_{2\alpha}$). Electron micrograph showing neoplastic cells with enlarged nuclei (*N*), nuclear inclusions, 2–3 nucleoli (*Nc*), red blood cells (*Rc*) and microvillous projections uranyl acetate and lead citrate, × 8,000).

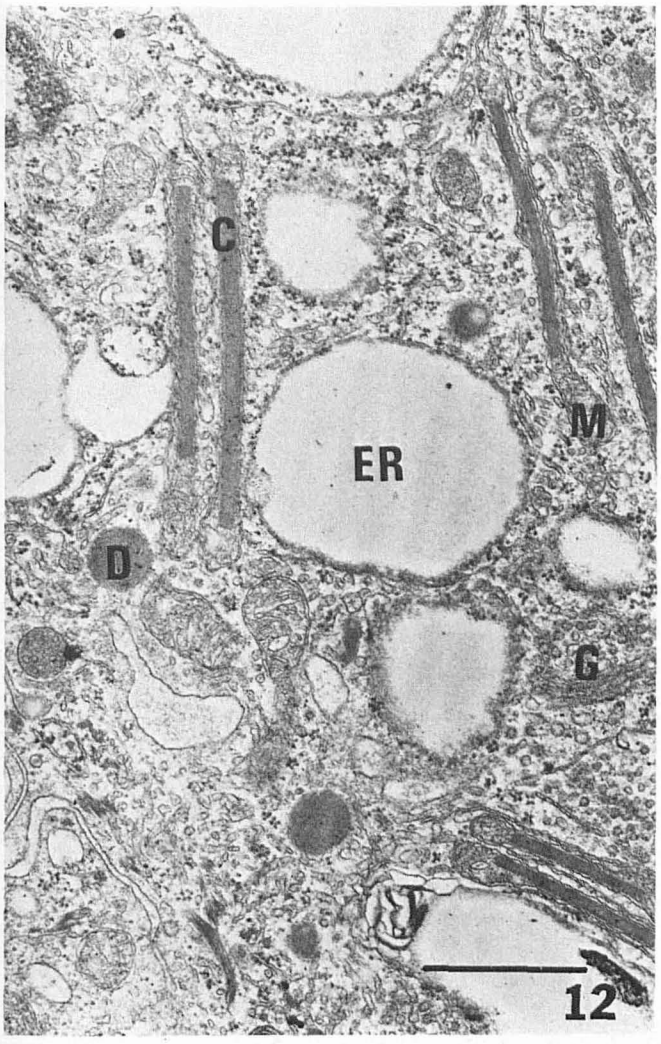

FIG 12. Basal cell carcinoma (3-methylcholanthrene & $PGF_{2\alpha}$). Several crystalloid formation (*C*) are visible within mitochondria (*M*) near endoplasmic reticulum cisternae (*ER*) and Golgi complex (*G*). Dense granules (*D*) can also be seen (uranyl acetate and lead citrate stain, × 20,000).

occurred in MCA and hydrocortisone-treated mice. Only a mild epidermal hyperplasia with few proliferative cells which are mainly acanthotic and vacuolated are visible (Fig 8). A marked inhibition of cell growth was also observed in MCA and hydrocortisone-treated rats. Here, only a slight epithelial hyperplasia with large vacuolated cells are present. An advanced inhibition of tumor development can also be seen in hypophysectomized and MCA-treated mice and rats. A slight epidermal hyperplasia and vacuolated cells with pyknotic nuclei are only visible. No histologic changes occurred in the skin of mice and rats treated with solvent systems alone.

Light Microscopic Autoradiography

Light microscopic autoradiography revealed significant changes of [^{3}H]-thymidine incorporation in squamous cell and basal cell carcinomas. A marked increase of labeled cells can be seen in mice with squamous cell carcinoma and concomitantly treated with $PGF_{2\alpha}$, where the reaction is mostly located at the periphery of tumor masses; no labeled cells are visible in the horn or epithelial pearls (Fig 9). An interesting autoradiographic pattern is observed in tumors of MCA and $PGF_{2\alpha}$-treated rats. It seems that there are 2 cellular compartments: one of dark cells, whose nuclei are heavily labeled and the second compartment of light cells with pale cytoplasm in which only very few nuclei are labeled (Fig 10). Several heavy labeled cells occurred in the squamous cell carcinomas of mice and solid basal cell carcinoma of MCA and thyroxine-treated rats. Only few labeled cells are seen in mice treated with MCA and hydrocortisone. Also a marked reduction of labeled cells can be seen in the MCA and cortisol-treated rats. Quantitative estimation of autoradiograms from carcinomas of mice and rats showed significant differences ($P < 0.001$) between experimental groups (Table II).

Ultrastructural Pathology

Interesting changes of cell organelle distribution in the carcinomas of mice and rats can be seen following different hormonal treatments. Thus, in squamous cell carcinoma of mice the hypertrophic dark and light cells are separated by widened intercellular spaces. Few desmosomes and several microvillous projections can be seen. In basal cell carcinomas induced in rats the ultrastructural pattern is different; neoplastic cells exhibit a large polysome population, productive ergastoplasm, and mitochondria. Nuclei are enlarged with nucleoli and only few tonofilaments are visible. A stimulation of cell growth and differentiation occurred in mice with squamous cell carcinoma following $PGF_{2\alpha}$ administration. Neoplastic cells with enlarged

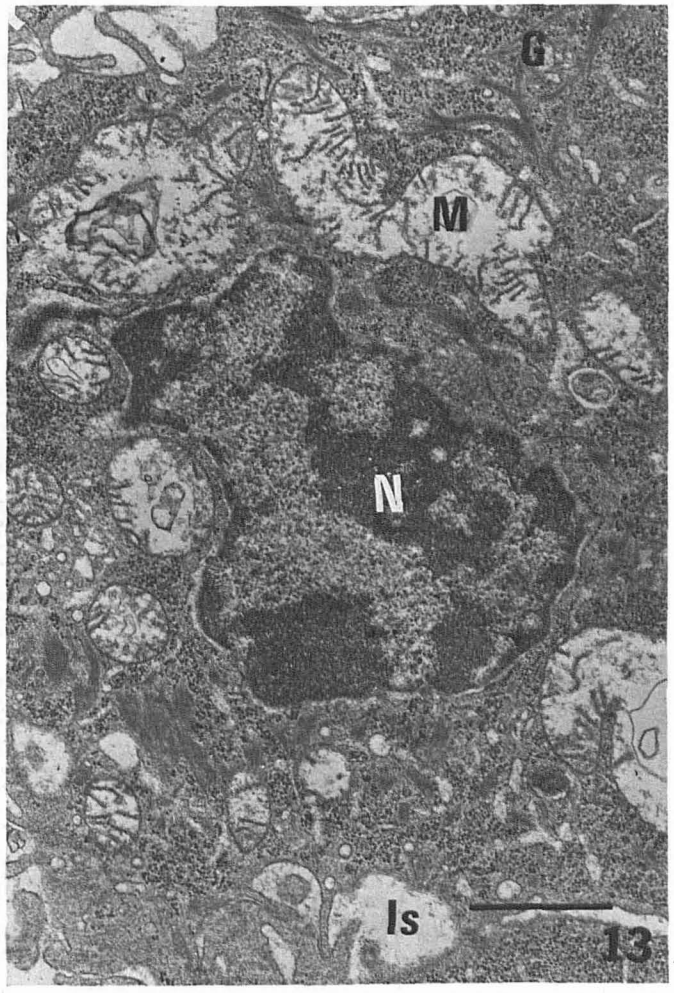

FIG 13. Squamous cell carcinoma (3-methylcholanthrene & thyroxine). Neoplastic cells exhibit a large population of hypertrophied mitochondria (*M*) with myelin figures, glycogen particles (*G*), nuclei (*N*) and enlarged intercellular spaces (*Is*) (uranyl acetate and lead citrate stain, × 18,000).

nuclei and nuclear inclusions and 2–3 nucleoli, mitonchondria, increased number of polysomes, dense granules, and tonofilaments are frequently seen. Intercellular spaces are enlarged and red blood cells can be seen (Fig 11). Also an increase of dark and light cells occurred in rats with basal cell carcinoma and treated with $PGF_{2\alpha}$. Neoplastic cells contain high electronic dense granules agglomerated near Golgi zone and enveloped by a membrane (lysosome-like structures), crystalloid formations, and few tonofilaments. Crystalloid-formations originate from mitochondria and are located near rough endoplasmic reticulum cisternae (Fig 12). A marked enhancement of cell growth occurred in squamous cell carcinoma following thyroxine administration. The dark and light cells contain large conglomerated cellular structures (phagolysosomes). Hypertrophied and ballooned mitochondria with cristae and lamellar concentric structures (or myelin figures) and an increased polysome and glycogen granule population are also observed (Fig 13). Dispersed and poorly differentiated cells connected only by cytoplasmic extensions are also visible. An increased accumulation of α-glycogen granules and vacuolation occurred in the neoplastic cells following thyroxine administration in rats. Several enlarged cells with hypertrophied nuclei and glycogen granules agglomerated in rosettes (α-glycogen particles) can frequently be seen (Fig 14). Sometimes, cytoplasm of neoplastic cells is occupied mainly by large vacuoles (lumina) pushing the nucleus to the periphery (Fig 15). Advanced keratinization with large areas of keratin and keratohyaline granules occurred following estradiol administration in mice and rats. At higher magnification, it is possible to see numerous intramitochondrial dense granules (proteins?) sometimes concentrate near the nuclei (Fig 16). They occurred in almost all specimens from this group. A marked increase of tonofilaments occurred in neoplastic cells following calcitonin administration in mice and rats. Cells are shrunken with small nuclei, scattered and small keratohyaline granules, and numerous bundles of clumped tonofilaments which occupy almost the entire cytoplasm can be seen (Fig 17). A marked inhibition of neoplastic cell growth occurred following hydrocortisone administration in mice. Epidermal cells are only slightly enlarged with shrunken and indentated nuclei, poor in chromatin. A marked inhibition of neoplastic cells occurred also in MCA and hydrocortisone-treated rats. Epidermal cells are slightly enlarged, with bundles of tonofilaments and mitochondria. Basement membrane is intact. A similar neoplastic inhibition is observed in hypophysectomized mice and rats treated with MCA. No ultrastructural changes were detected in the skin of mice and rats treated with solvent systems alone.

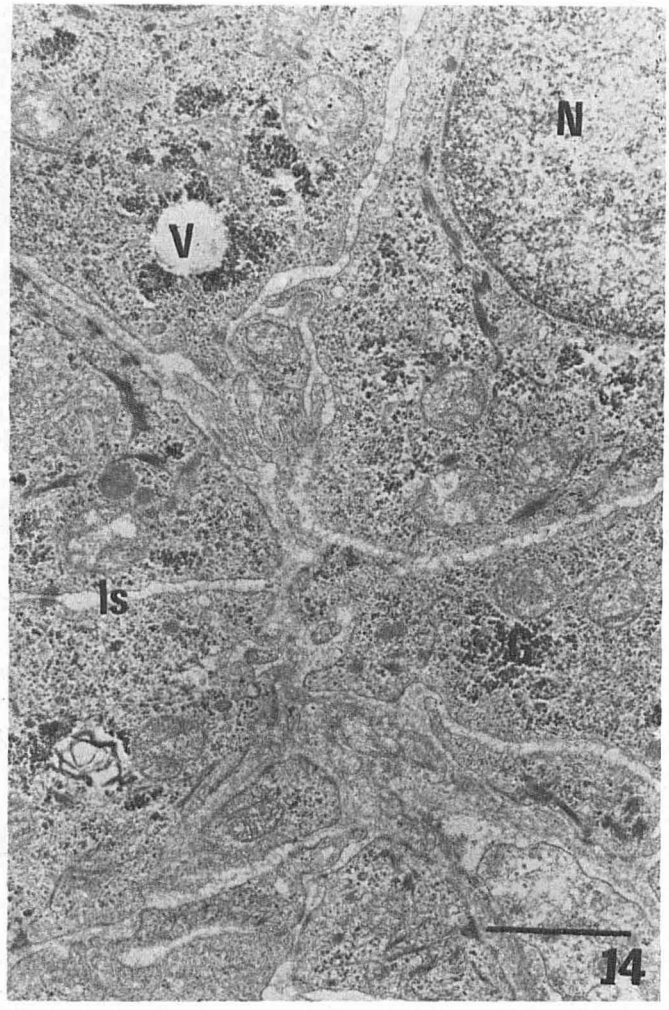

FIG 14. Basal cell carcinoma (3-methylcholanthrene & thyroxine). Marked cellular stimulation, abundant glycogen synthesis (*G*), hypertrophic nuclei (*N*) and vacuoles (*V*) can be seen. Cells are separated by narrow intercellular spaces (*Is*) and cell extensions (uranyl acetate and lead citrate stain, × 18,000).

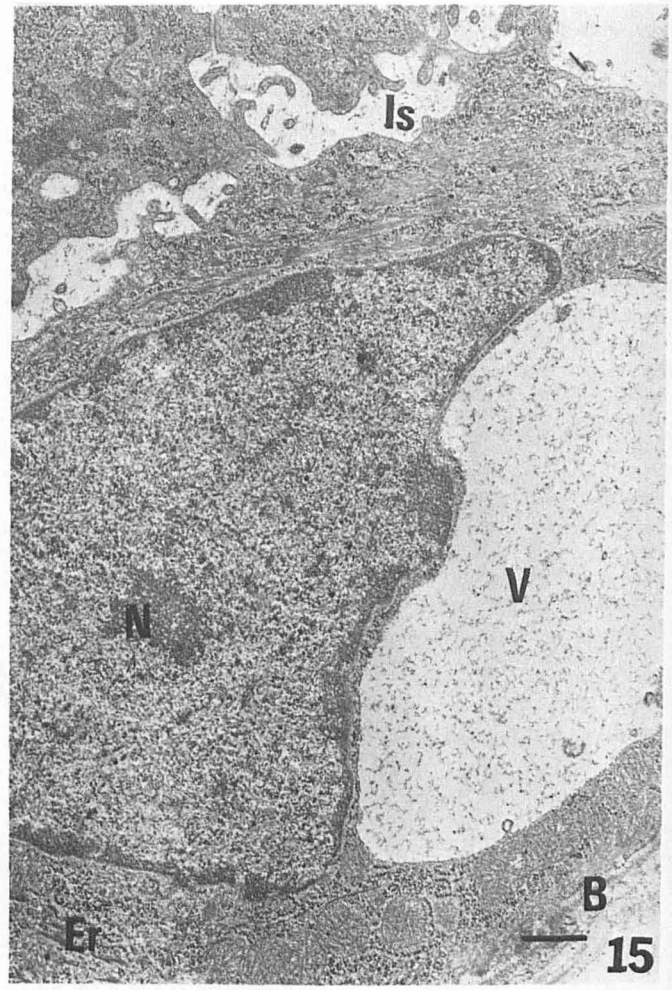

FIG 15. Basal cell carcinoma (3-methylcholanthrene & thyroxine). Large vacuoles (*V*) or lumina occur in the cytoplasm of neoplastic cells. These cells exhibit an extensive endoplasmic reticulum (*Er*), hypertrophic nuclei (*N*). Basement membrane (*B*) and enlarged intercellular spaces (*Is*) are also visible (uranyl acetate and lead citrate stain, × 8,000).

Electron Microscopic Autoradiography

Electron microscope autoradiography revealed some interesting findings regarding the site(s) of DNA synthesis and its

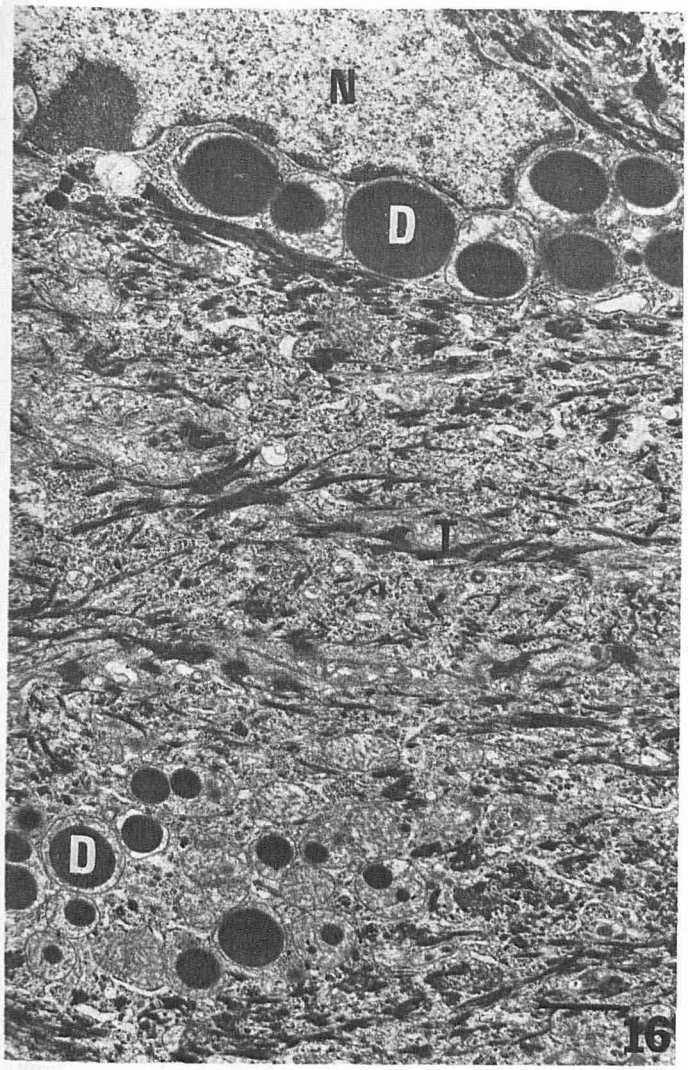

FIG 16. Squamous cell carcinoma (3-methylcholanthrene & estradiol). Neoplastic cells exhibit a large population of intramitochondrial granules (*D*), tonofilaments (*T*) and nuclei (*N*) (uranyl acetate and lead citrate stain, × 12,000).

repartition in the neoplastic cells following hormone administration. Thus, an increased [^{3}H]-thymidine incorporation seen as developed grains occurred over the nuclear chromatin of squamous cells of mice treated with MCA and thyroxine. Distribution of [^{3}H]-thymidine over the nuclear membrane can be seen in the neoplastic nuclei of MCA and estradiol-treated mice. Here, the developed grains are mostly located over the peripheral chromatin and nuclear envelope (Fig 18).

Scanning Electron Microscopy

Scanning electron microscopy revealed salient features regarding the cytoarchitecture, orientation, and cell surface of neoplastic cells. Thus, neoplastic squamous cells of MCA and thyroxine-treated mice appeared polymorphic. Some are triangular or pear-shaped (pyriformis) exhibiting small blebs and ruffles on their surface. Cells are separated by enlarged intercellular spaces and connected by an extensive network of microvilli and collagen fibrils (Fig 19). SEM of basal cell carcinomas of rats treated with MCA and thyroxine revealed more regular and oval cells with flat surfaces and only few blebs; some cells are cylindroid and elongated, orderly disposed with few blebs. They are separated by enlarged intercellular spaces and cell extensions (Fig 20). Estradiol administration in mice and rats induced a significant increase in keratinization. Frequently, all keratin layers are agglomerated in concentric sheets (onion sheet-like).

DISCUSSION

The present findings clearly demonstrate that hormones or hormone-like substances such as thyroxine, prostaglandins, estradiol, hydrocortisone, calcitonin, or hormone deprivation (hypophysectomy and gonadectomy), can markedly change the onset and development of carcinomas induced by a long-term administration of a known chemical carcinogen (3-MCA) in mice and rats. Thus, thyroxine, $PGF_{2\alpha}$, estradiol, calcitonin, and PGA_2 significantly accelerated the onset and development of squamous cell carcinomas in mice and basal cell carcinomas in rats, whereas hydrocortisone, hypophysectomy, and gonadectomy inhibit it. The histologic features are accompanied by significant changes in DNA synthesis, ultrastructure, and cell surfaces of cancerous cells. Because hormones are widely used in the treatment of different types of cancers or leukemic states—alone or combined with cytostatic agents or radiation therapy—we would like to review the effects of hormones on neoplastic growth in target tissues (hormone-dependent can-

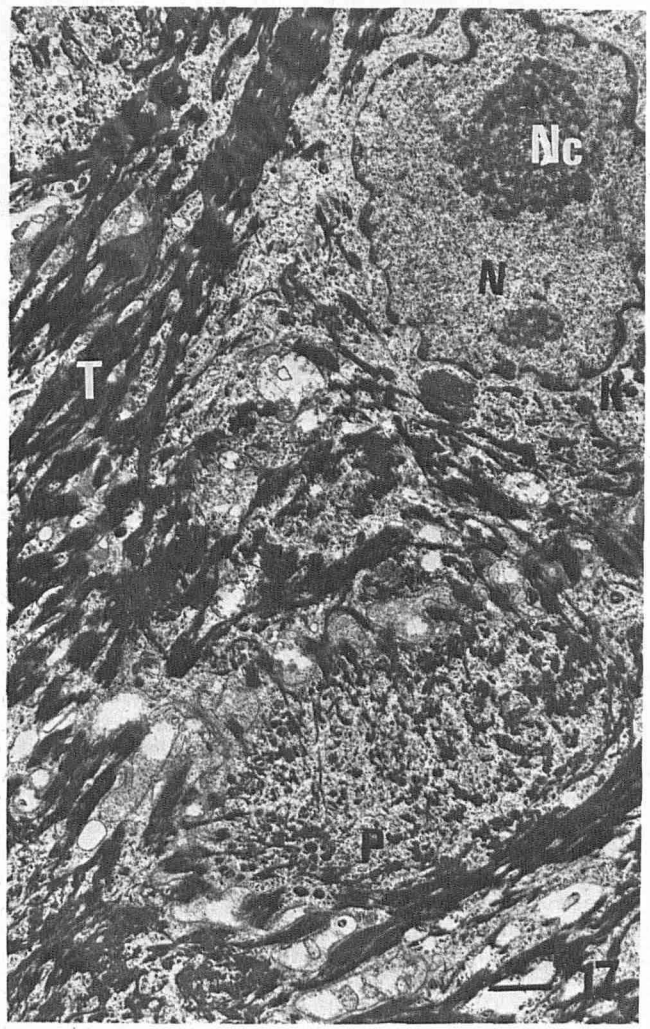

FIG 17. Squamous cell carcinoma (3-methylcholanthrene & calcitonin). Cytoplasm of neoplastic cells is almost entirely replaced by a marked increase of tonofilaments (*T*), which appear as clumped bundles; polysomes (*P*), small keratohyaline granules (*K*), nuclei (*N*) and nucleoli (*Nc*) are also seen (uranyl acetate and lead citrate stain, × 8,000).

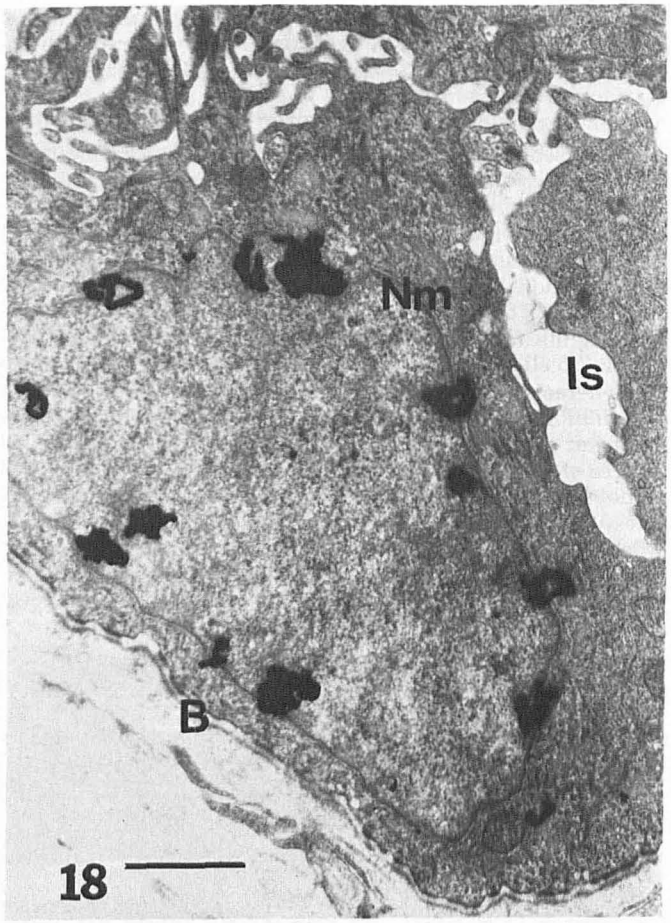

FIG 18. Electron microscope autoradiogram of 3-methylcholanthrene & estradiol-treated mouse showing a peripheral distribution, namely on the nuclear membrane (*Nm*) of [^{3}H]-thymidine as developed grains. No grains are visible over basement membrane (*B*) or intercellular spaces (*Is*). Ilford L_4 nuclear emulsion, exposure 3 mo (uranyl acetate and lead citrate stain, 15,000).

cers), on nontarget tissues including skin, possible mechanism(s) of action on epidermal neoplastic cells and therapeutic implications.

Most previous investigations have been carried out to study the hormone effects on hormone-dependent cancers (carcinomas of the breast, prostate, or endometrium) [10]. Thus, progesterone administration caused an increased tumor incidence by shortening the latent period of experimental mammary cancer [11]. Other hormones e.g., prolactin or insulin or thyroid hormone deficiency also exert an important role in the neoplastic growth of mammary glands [12–14]. Androgens can influence the development of mammary carcinoma [15], as well as prostatic tumors [16].

The influence of hormones or hormone-like substances on nonhormone dependent organs (liver, skin) has been described only by few authors. Liver tumors induced by ethylnitrosourea administration occurred more frequently in male than in female mice [17]. Male mice are also more susceptible than females to skin tumors initiated by a small dose of DMBA and promoted by croton oil [18]. Hormone-like substances such as epidermal growth factor (EDG) and chalones can also influence the skin tumor development in mice. EGF was found to enhance the induction of mouse skin tumors after MCA application whereas, epidermal chalones (G_2) inhibit it [19].

Are the hormones tumor promoters, cocarcinogens, or do they act directly on the cells rendering them more susceptible to carcinogens? At present, there is no evidence that hormones are primary carcinogens. However, there is ample evidence that hormones notably influence the course of neoplastic process in the target tissues, probably by a preparative action of the cells for carcinogens, acting as tumor promoters [20]. The present investigations revealed that squamous cell and basal cell carcinomas induced by a chronic administration of a chemical carcinogen (3-MCA) are significantly responsive to hormones or hormone-like substances which can act as cocarcinogens rendering the cells more susceptible to carcinogens and thus, shortening the latent period. Hydrocortisone, hypophysectomy, and gonadectomy exert the opposite effect. Light and electron microscopic autoradiography revealed marked changes in DNA synthesis and its repartition and consequently neoplastic transformation is substantially influenced. Hence, the present data suggest that hormones exert their influence on neoplastic cells by inhibiting DNA synthesis, especially in the S-phase of the cell cycle. Ultrastructural studies revealed that the cellular pattern is shifted by hormones, toward a poorly differentiated type, well differentiated or only dysplastic one. Hormones also induced marked mitochondrial alterations as in thyroxine-treated mice or appearance of numerous crystalloid-formations in neoplastic cells of $PGF_{2\alpha}$-treated rats. Scanning electron microscopy showed that hormones induced significant changes on the neoplastic cell surfaces (blebs, ruffles).

From the present investigations it appears that hormones notably change and thus can modulate the neoplastic transfor-

FIG 19. SEM revealing at higher magnification the blebs (*B*) and ruffles (*R*) on the squamous neoplastic cell surfaces (3-methylcholanthrene & thyroxine). Cells are connected by several microvilli (*Mv*) and cell extensions (*CE*) (critical point CO_2 method, gold coated × 8,000).

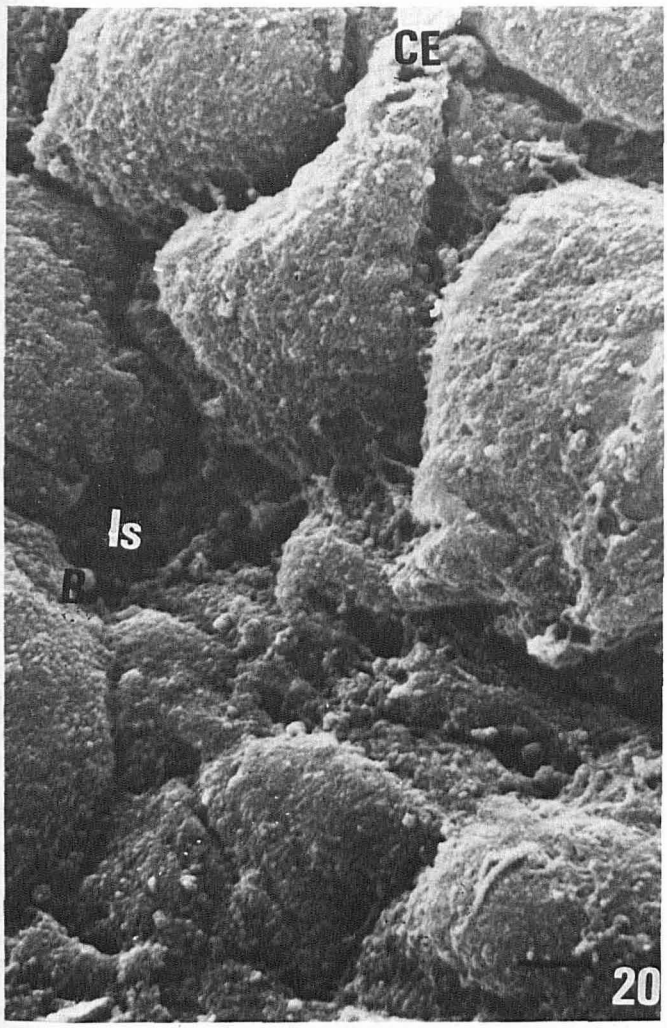

FIG 20. Scanning electron microscopy of basal cell carcinoma (3-methylcholanthrene & thyroxine). At higher magnification, polyhedral neoplastic cells exhibit small blebs (*B*) and ridges on the surface. Some are spindle-shaped and emit long extensions (*CE*). Enlarged intercellular spaces (*Is*) (critical point method, gold coated, × 8,000).

mation acting as "modulators" of epidermal carcinogenesis. Although squamous cell carcinoma and basal cell carcinoma are not hormone-dependent tumors, they are responsive to hormone administration and this can be a useful experimental model for the study of carcinogenesis in general and its responsiveness to hormone therapy. It is also possible by changing the "hormonal milieu" to markedly influence the cellular evolution of epidermal cancers. Since these tumors are identical to those occurring in man, these findings may suggest that hormones have important therapeutic implications in humans.

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